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Effects of Tree Root-Derived Substrates and Inorganic Nutrients on Pyrene Mineralization in Rhizosphere and Bulk Soil

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ABSTRACT

This study investigated the effects of organic and inorganic nutrients on the microbial degradation of the common soil contaminant pyrene. The material used in this investigation was collected from potted trees that had been growing for over a year in a soil artificially contaminated with polycyclic aromatic hydrocarbons. Soil was removed from the nonroot (bulk) and root (rhizosphere) zones of these pots and used in mineralization studies that tracked microbial degradation of ^{14}C-pyrene. The factors influencing degradation in these zones were then tested by amendment with essential inorganic nutrients or with root-derived materials. As expected, pyrene mineralization was greater in soil removed from the rhizosphere than in bulk soil. The rate of mineralization in rhizosphere soil was inhibited by inorganic nutrient amendment, whereas nutrients stimulated mineralization in the bulk soil. Pyrene mineralization in bulk soil was also increased by the addition of root extracts intended to mimic exudation by living roots. However, amendment with excised fine roots that were allowed to decay over time in soil initially inhibited mineralization. With time, the rate of mineralization increased, eventually exceeding that of unamended bulk soil. Combined, the initial inhibition and subsequent stimulation produced a zero net impact of decaying fine roots on bulk soil mineralization. Our results, in conjunction with known temporal patterns of fine root dynamics in natural systems, support the idea that seasonal variations in nutrient and substrate availability may influence the long-term effect of plants on organic degradation in soil, possibly reducing or negating the beneficial effects of vegetation that are often observed in short-term studies.

Root-derived carbon substrates, released during exudation and during the decay of fine roots, are important sources of plant influence on microbial communities in the rhizosphere. These root-derived substrates are often implicated in explaining enhanced microbial degradation of persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAHs), in soil. Indirect support for this comes from findings that PAH dissipation and counts of microbial PAH degraders increase with proximity to plant roots (Joner and Leyval, 2003). In addition, POP-degrading microorganisms are capable of utilizing root-derived carbon substrates as a sole energy source (Leigh et al., 2002; Rentz et al., 2004), potentially increasing their ability to colonize and function in the rhizosphere.

Relatively little work has been done to directly test the effect of root exudates or fine root decay on microbial degradation of PAHs. While several studies have shown that root exudates (or compounds that mimic exudates) can enhance mineralization or dissipation of PAHs (Yoshitomi and Shann, 2001; Joner et al., 2002), others report no effect (Wetzel et al., 1997) or even inhibition (Kamath et al., 2004; Qiu et al., 2004; Rentz et al., 2004). The impact of fine root decay on microbial degradation of PAHs has rarely been tested (Parrish et al., 2005). If both root exudation and decay increase the microbial degradation of PAHs, the perennial nature and size of tree root systems would be advantageous for phytoremediation of PAH-contaminated soils. However, seasonal root dynamics exhibited by temperate trees (Tierney et al., 2003; Mueller and Shann, 2006) could lead to variable effects on PAH degradation in soil. The few experiments aimed at using trees to remediate PAH contamination have had mixed results (Liste and Alexander, 2000; Qiu and Loehr, 2002; Genney et al., 2004; Tang et al., 2004; Mueller and Shann, 2006).

Other gradients between bulk soil and the rhizosphere may also impact POP dissipation. Concentrations of nitrogen, phosphorus, and other essential inorganic nutrients can differ widely between rhizosphere and bulk soils, with rhizosphere soil often being relatively nutrient-depleted (Wang et al., 2001; Wang et al., 2004). Since microbes and plants are known to compete for soil nutrients such as nitrogen (Hodge et al., 2000), it is clear that these gradients may also be important in determining the outcome of phytoremediation trials. For example, in a field trial, mineralization of phenanthrene (a common PAH) in planted soil was increased relative to unvegetated soil only after amendment with nitrogen and phosphorus (Siciliano et al., 2003). This was attributed to nutrient limitation of microbial PAH degraders in the planted soil. Even in the absence of plants, PAH degradation is often limited by inorganic nutrient availability (Breedveld and Sparrevik, 2000; Joner et al., 2002) although this is not always the case (Carmichael and Pfaender, 1997; Johnson and Scow, 1999). Despite our minimal understanding regarding the effect of inorganic soil nutrients on POP dissipation in planted soils, planted and unplanted treatments in phytoremediation trials are often fertilized.

The purpose of this study was to investigate the influence of living and decaying fine roots of red maple (*Acer rubrum*) on microbial degradation of a model PAH, pyrene. Specifically, we tested: (1) the ability of microbial communities in rhizosphere and bulk soils to mineralize freshly added pyrene, and (2) the impacts of

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Abbreviations: POPs, persistent organic pollutants; PAHs, polycyclic aromatic hydrocarbons.

root extracts, decaying fine roots, and essential inorganic nutrients on microbial pyrene mineralization.

MATERIALS AND METHODS

Experimental Materials

Basic characterization of the experimental soil was conducted by the Agricultural Analytical Services Laboratory of Pennsylvania State University. Classified as a silty clay loam, the soil had a particle size distribution of 19.1% sand, 53.2% silt, and 27.7% clay and was 1.8% organic matter. The pH was 7.7 and the CEC 17.3 $cmol_c\ kg^{-1}$. Nutrient levels were as follows: 29.8 mg kg^{-1} nitrate nitrogen, 2.5 mg kg^{-1} ammonium nitrogen, 52.5 mg kg^{-1} phosphate, 24.2 $cmol_c\ kg^{-1}$ calcium, and 0.2 $cmol_c\ kg^{-1}$ potassium. Field capacity was determined to be 47%.

For this and a related study (Mueller and Shann, 2006) the loam was artificially contaminated with PAHs, potted, planted, and maintained in a greenhouse for over a year. For details regarding procedures used to contaminate soil with PAHs, and to maintain planted pots, see Mueller and Shann (2006). Briefly, soil was brought to a final concentration of 300 mg kg^{-1} for each of three PAHs (phenanthrene, anthracene, and pyrene). This was done by adding a concentrated PAH solution to a small volume (1 kg) of soil and then mixing this contaminated soil with a larger volume (12.5 kg) of 'clean' topsoil. Mixed soil was then placed into 17-L greenhouse pots. Analysis of replicate soil samples from each pot indicated that PAHs were uniformly distributed in the soils at the concentrations intended. Potted soils were allowed to age with the contaminants for 1 mo before planting. After this period, a single 1-yr-old red maple (Sylva Native Nursery, PA) sapling was planted into each of five replicate pots. Greenhouse air temperatures ranged from −4 to 39°C and soil temperatures from 1 to 29°C. No supplemental lighting was provided and soils were not fertilized. After the first growing season, each tree dropped its leaves and went through a period of dormancy. Trees resumed growth in the spring and displayed typical growth throughout the next growing season.

Soil Sampling

Contaminated pots were destructively sampled in December, approximately 15 mo after planting and before leaf senescence. Soil was removed from each pot and manually processed. Bulk soil (soil not colonized by roots) was immediately separated and set aside. The remaining soil consisted of small to relatively large clumps clustered around fine roots. Soil clusters that were only loosely bound to roots or that did not contain root networks within them were discarded. The remaining soil was carefully removed from the root system and considered rhizosphere soil. Collected soils (bulk and rhizosphere) were sieved to 4.75 mm, placed in sterile tubes, and stored at room temperature until used in ^{14}C-pyrene mineralization studies. The moisture content of soils following the sampling procedure ranged from 11 to 16%.

Plant Harvest

Tree roots were separated from their shoots. Red maple root systems were comprised mostly of networks of lighter colored fine roots attached to a few dark coarse ones. Roots were washed with a mild detergent and then thoroughly rinsed. Intact root systems were kept at 4°C until used to prepare root amendments (described below). After known amounts were removed for root amendments, the remaining roots and shoots were dried in an oven to a constant weight. Root and shoot measurements were used to examine possible relationships between plant biomass and PAH mineralization.

Root Amendments

Root extract and whole root amendments were prepared from red maple trees within 48 h of destructive harvesting. Only fine roots (first-, second-, and third-order) (Pregitzer et al., 2002) still attached to the tree were used. Extract and whole root amendments were made from each of the five red maple replicates. After being removed from the root system the roots were rinsed in distilled water and blotted dry.

Whole root amendments were intended to mimic natural fine root death and decomposition. Translocation of nutrients to senescing roots is not thought to be significant (Gordon and Jackson, 2000). Thus amendment with artificially excised fine roots should realistically simulate natural root decay and the release over time of their organic and inorganic constituents.

Root extract amendments were intended to mimic the release of compounds by living roots. Extract amendments were prepared by extraction of fresh roots according to the methods of Kamath et al. (2004) and Rentz et al. (2004). Carbon sources known to be present in tree root exudates have been quantified and identified in the extracts produced by this method (Rentz et al., 2004). Root tissue (0.02 g per tree) was ground with a mortar and pestle in 3 mL of distilled water and homogenized (Brinkman Polytron Homogenizer) with an additional 3 mL of distilled water for 30 s. The resulting suspension, along with 4 mL of rinse water, was first filtered (No. 3 Whatman paper) and then filter sterilized using presterilized Nalgene filterware. Excised whole roots and root extracts were stored at 4°C until used in mineralization studies.

Analysis of Root Extracts Used as Root Exudate Amendments

A limited amount of characterization has been done on root extracts from similar studies (Kamath et al., 2004; Rentz et al., 2004; Rentz et al., 2005). For broad comparison, root extracts were analyzed here for their total organic carbon (TOC) content and by electrospray ionization mass spectrometry (ESI-MS) for the mass distribution of component compounds. Total organic carbon was estimated using the colorimetric method of Heanes (1984). One mL of root extract from each tree was digested with 1 mL concentrated sulfuric acid and 1 mL potassium dichromate ($K_2Cr_2O_7$) at 150°C for 30 min. After cooling, 3.5 mL water was added to each sample and absorbance determined at 600 nm. The TOC in each sample was calculated against a standard curve for D-glucose. Extracts from two separate trees, one at the low end of observed TOC values and one at the upper end, were analyzed by ESI-MS. Extracts were diluted 1:5 (extract/solvent) using a 50% aqueous acetonitrile solution. Formic acid (1%) was used as the electrospray buffer for introduction into the ESI-MS (model Q-T/2 mass, Micromass UK) at a flow rate of 5 μL min^{-1}. Data acquisition and processing was accomplished with MassLynx (4.0).

^{14}C-pyrene Mineralization in Soil

The ability of rhizosphere and bulk soil microbial communities to mineralize ^{14}C-pyrene was measured using serum bottle radiorespirometry (Knaebel and Vestal, 1988). Soil samples were homogenized with a mortar and pestle before 5-g (fresh wt.) aliquots were placed in glass serum bottles. The radiotracer (Sigma, 4,5,9,10 ^{14}C-pyrene) was added to each bottle (as a 50 μL acetone solution, 515 Bq ^{14}C or 103 Bq g^{-1}

soil). Bottles containing rhizosphere or bulk soil were prepared for each of the five destructively harvested pots (and trees) so that the various treatment groups ended up with five replicates (one bottle from each pot and/or tree). Experimental treatments included unamended bulk and rhizosphere soils, bulk and rhizosphere soils amended with inorganic nutrients, and bulk soils amended with root extracts or excised fine roots. Depending on the treatment, bottles were then amended with 1 mL sterile water, 1 mL sterile nutrient solution (containing 250 mg l^{-1} of both ammonium nitrate and potassium phosphate), 1 mL root extract, or 0.02 g excised fine roots plus 1 mL sterile water. Sterile water and nutrient treatments were given to both rhizosphere and bulk soils, but only bulk soils were treated with the root extract and whole root amendments. Root amendments were prepared from the tree associated with the specific bulk soil being treated. After nutrient and root amendments were added, each bottle was vortexed and stored at room temperature in the dark. Mineralization setup was completed within 48 h after destructive sampling. The CO_2 released during subsequent microbial ^{14}C-pyrene mineralization was captured on KOH-saturated wicks suspended in each bottle. Wick radioactivity was measured by liquid scintillation (Packard 2200 CA Tri Carb liquid scintillation analyzer). Wicks were periodically removed, replaced, and counted for 1 to 45 d or 1 to 170 d after the addition of ^{14}C-pyrene. Select bulk soil treatments, including unamended and root extract and excised root-amended soil, were maintained and counted for the extended period to assess long-term effects of these different root-derived materials on microbial activity. Extended study serum bottles were respiked with ^{14}C-pyrene (580 Bq) at 110 d.

Data Analysis

Paired *t* tests were used to evaluate and report differences between treatments on specific dates. Potential relationships between plants and PAH dissipation in potted soil or pyrene mineralization in serum bottle studies was explored using linear regression and correlation. All statistical tests were performed using SYSTAT 10.2 software. Significance of treatment effects was evaluated using $\alpha = 0.05$. Tests with p values less than 0.1 are described as having marginal significance.

RESULTS

Pyrene Mineralization

Prior contact with red maple roots increased later mineralization in soil. Figure 1 shows the pyrene mineralization curves for rhizosphere and bulk soil after collection from greenhouse pots. By Day 45, mineralization in rhizosphere soil was 17% higher than in bulk soil ($p < 0.05$, paired *t* test). This difference was largely due to the higher initial (Days 1 through 7) rate of mineralization in rhizosphere soil. For equivalent time periods, mineralization rates (of treatments) can be compared using the slope of the linear response (Table 1). In rhizosphere soil, the initial slope (5.90) was significantly higher than that of the unamended bulk soil (4.96).

For the first 2 mo of the study, bulk soil mineralization in serum bottles was positively related ($p < 0.05$, $R^2 = 0.87$) to the biomass of roots in the greenhouse pot from which it was collected. Rhizosphere soil was not.

The effect of inorganic nutrient amendments on pyrene mineralization in soil also depended on the history of exposure to tree fine roots. Inorganic nutrient

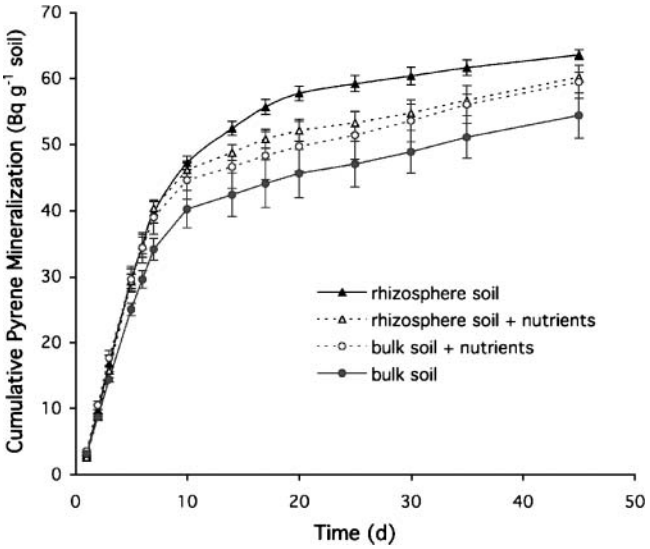

Fig. 1. Inorganic nutrient effects on ^{14}C-pyrene mineralization in soils originally collected from greenhouse pots contaminated with polycyclic aromatic hydrocarbons (PAHs) and planted with red maple. Mean cumulative mineralization was calculated from the radioactivity of $^{14}CO_2$ evolved from the replicate bottles ($n = 5$) within each treatment (rhizosphere or bulk soil, amended or not with inorganic nutrients). Since the initial concentration of radioactivity was 103 Bq g^{-1} soil, the graphed values for cumulative mineralization are approximately equivalent to percentage mineralization.

amendment significantly decreased the final extent of pyrene mineralization in rhizosphere soil ($p < 0.05$, paired *t* test, Fig. 1). By Day 45, mineralization in nutrient-amended rhizosphere soil was reduced by 5% relative to unamended rhizosphere soil. This reduction was due to the lower rate of mineralization that occurred in the nutrient-amended soil between Day 10 and 20. In bulk soil, the addition of nitrogen and phosphorus initially increased (slightly) the rate of pyrene mineralization (Days 1 through 7, Table 1) relative to unfertilized bulk soil. Rates in both dropped significantly after 7 d. The 9% difference between treatment means (at Day 45) was not statistically significant.

Root extract and excised fine root amendments had different effects on microbial mineralization. Bulk soil amended with root extracts mineralized more pyrene than either unamended or excised root-amended soils (Fig. 2 and 3). The final extent of mineralization in soil amended with root extracts was 12% higher than the other treatments ($p < 0.05$, paired *t* test). This final extent was due primarily to the greater rates of mineralization that occurred within the first 7 d of the study (Table 2). Amendment of bulk soil with excised red maple fine roots did not significantly alter the final extent of pyrene mineralization relative to the unamended treatment. However, the effect of decaying fine roots on microbial pyrene mineralization did have a temporal component. Through the first 30 d of the mineralization experiment, amendment with excised fine roots resulted in a marginally significant reduction of pyrene mineralization by 17% ($p < 0.1$, paired *t* tests). Over time, soils amended with excised roots then exhibited greater rates of pyrene mineralization than unamended controls (Table 2). By the end of the study there was no dif-

Table 1. Linear regression of cumulative pyrene mineralization and time for soil amended with inorganic nutrients or left unamended. Separate regressions were performed on distinct phases of mineralization (apparent in Fig. 1 and verified by statistical comparisons of linear parameters). When no significant differences were found between amended and unamended treatments (within a soil type), data was pooled for regression analysis. All one-way ANOVA models were significant ($p < 0.001$).

	Parameter estimates for the linear equation: cumulative pyrene mineralization = slope (days) + intercept			
Soil type	**Soil data**	**Slope ± SE†**	**Intercept ± SE**	**R^2**
Rhizosphere soil				
Days 1 through 7	**pooled**	**5.90 ± 0.11**	**−1.92 ± 0.48**	**0.98**
Days 10 through 20	**unamended**	**1.04 ± 0.14**	**36.00 ± 2.16**	**0.76**
	nutrient-amended	**0.54 ± 0.17**	**39.03 ± 2.64**	**0.41**
Days 20 through 45	**pooled**	**0.39 ± 0.04**	**44.07 ± 0.97**	**0.59**
Bulk soil				
Days 1 through 7	**unamended**	**4.96 ± 0.13**	**−1.11 ± 0.55**	**0.98**
	nutrient-amended	**5.71 ± 0.22**	**−0.94 ± 0.96 (NS)‡**	**0.95**
Days 10 through 45	**pooled**	**0.40 ± 0.06**	**35.77 ± 1.48**	**0.25**

† SE, standard error.
‡ NS, not significant.

ference between the extent of cumulative pyrene mineralization in unamended bulk soil and excised root-amended bulk soil; 51% of the added ^{14}C-pyrene was mineralized in each. Root extract-amended soil mineralized 57% of added ^{14}C-pyrene.

Root Extract Characterization

Total organic carbon levels in the root extracts exhibited a range of 212 to 406 mg l^{-1} and are typical of values previously reported for root extracts and exudates (Rentz et al., 2004). The TOC was not correlated to the final extent of pyrene mineralization in bulk soil amended with root extracts ($p = 0.14$, $R^2 = 0.55$). Electrospray ionization-mass spectrometry analysis of root extracts shows that the majority of extract components ranged in molecular weight from 100 to 700 mass units and contained very few peaks greater than 1000 mass units (regardless of initial TOC in the extract [Fig. 4]).

DISCUSSION

Comparison of ^{14}C-pyrene mineralization in rhizosphere and bulk soils in this study supports the commonly reported 'rhizosphere effect', in which plant roots enhance dissipation of PAHs in soil by one of several possible mechanisms. Further, the positive correlation between bulk soil mineralization and root biomass loosely suggests that the effect of plant roots on pyrene mineralization may extend beyond soil that is tightly bound to roots. Other authors have also found evidence in support of this possibility (Tang et al., 2004). While prior contact with roots clearly increased pyrene mineralization in serum bottles, there was no difference between bulk and rhizosphere concentrations of phenanthrene, anthracene, or pyrene in soil removed from planted pots (data not shown) and PAH recovery was not related to any measure of plant growth or biomass. In a previous study utilizing the same soil and experimental conditions, similarly extensive PAH loss was

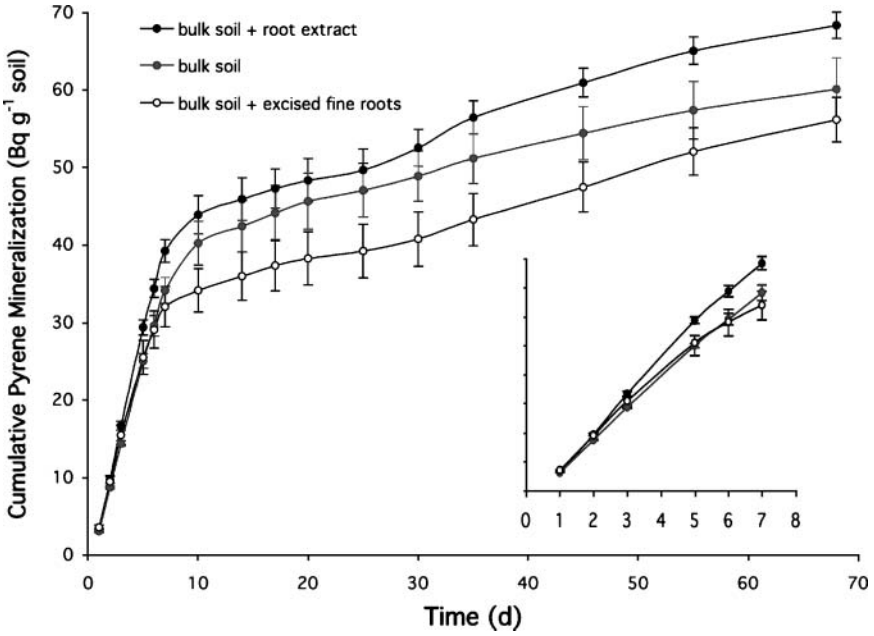

Fig. 2. Initial effects of root amendments on pyrene mineralization in bulk soil. Each replicate received an amendment of ^{14}C-pyrene (103 Bq g^{-1} soil). For visual purposes, the first 7 d are expanded as an inset.

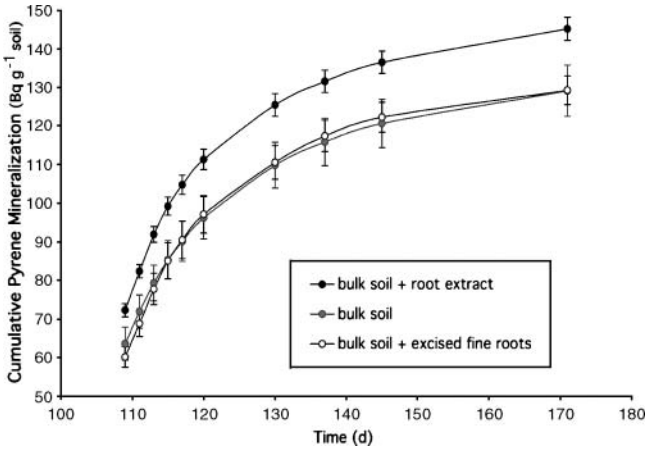

Fig. 3. Extended effects of root amendments on pyrene mineralization in bulk soil (see Fig. 2 for initial effects and study detail). On Day 110 of the study each replicate received a fresh amendment of ^{14}C-pyrene (116 Bq g^{-1} dry soil).

observed and attributed to high bioavailability and microbial degradation in the soil, regardless of the presence or absence of trees (Mueller and Shann, 2006).

If fine root dynamics and root-microbe interactions are constant through time, then the positive rhizosphere effects observed in mineralization studies (representing only a single time point) should translate into long-term positive rhizosphere effects on pyrene dissipation in planted soil. Variable responses to two separate sources of root-derived carbon, exudation, and fine root decay may have contributed to the lack of an observed planting effect on PAH dissipation in potted soil in our studies. In the mineralization study, experiments intended to mimic the effects of exudation and fine root death and decomposition revealed that these two important sources of root-derived carbon have different effects on pyrene degradation by microbes. Amendment of bulk soil with root extracts significantly increased pyrene mineralization. This result is not surprising, as numerous other studies (Yoshitomi and Shann, 2001; Joner et al., 2002) have shown that actual and artificial root exudates enhance PAH degradation.

Although it has also been suggested that natural fine root death and decomposition may also enhance PAH degradation (Olson et al., 2001; Leigh et al., 2002), direct support for this hypothesis is lacking. In this study, amendment of bulk soil with excised, whole red maple fine roots initially reduced pyrene mineralization by more than 15%. Previous work in our lab showed similar decreases in pyrene mineralization after amendment with excised fine roots of red mulberry trees (Mueller and Shann, 2006). Over time, pyrene mineralization in soils amended with excised red maple roots recovered so that by the end of the study there was no difference between unamended and excised root-amended treatments. Such variable effects of decaying roots on microbial degradation of organic contaminants are expected since decomposition is a dynamic process involving changes in the forms of available carbon substrates and dominant microbial communities over time (Wagner and Wolf, 1998).

In generating the soil used in our mineralization studies, potted soil and trees were allowed to experience seasonal changes in daylight, temperature, and activity—which would also be the case in field applications of phytoremediation at actual contaminated sites. Since exudation is highest at the root tip (Grayston et al., 1996) and root growth has strong seasonal patterns (Leigh et al., 2002; Tierney et al., 2003) the quantity of exudates released over time will vary seasonally. Fine root mortality also follows strong seasonal patterns with peaks of activity that are separate from those of fine root growth (Leigh et al., 2002, Tierney et al., 2003). Though root dynamics were not measured in this study, significant growth and death of fine roots likely occurred in potted plants despite the limited age and size of the trees. Red mulberry saplings have been shown to experience significant root growth and death throughout the length of just one growing season (Leigh et al., 2002). In addition, soil microbial communities in planted ecosystems have been shown to have seasonal shifts in community composition and activity (Smalla et al., 2001). These patterns, along with the results of our mineralization experiment and pot studies, suggest that different microbial responses to seasonally variable sources of root-derived carbon substrates could influence the outcome of long-term phytoremediation trials. The net effect of planting should partially be determined by the balance of root growth and decay over time. Notably, many studies are conducted over short durations and under constant environmental conditions and thus extrapolation of results to more natural settings is problematic.

In this study, differences in the quantity of carbon supplied in root amendments do not appear to drive the observed patterns of pyrene mineralization. The TOC levels in the root extracts were not significantly related to pyrene mineralization. Further, if microbial pyrene mineralization is related to the availability of carbon substrates in a simple linear fashion, then pyrene mineralization would have been expected to be highest in excised root-amended soil due to greater levels of carbon substrates added in this treatment. Although the TOC of excised root amendments was not measured, root extracts represent only an aqueous fraction of the same mass of fine roots used in excised root amend-

Table 2. Slope estimates from linear regression analysis of cumulative pyrene mineralization and time in bulk soil amended with root-derived material or left unamended. Separate linear regressions were performed for distinct phases of mineralization (apparent in Fig. 2 and 3). All ANOVA models were significant ($p < 0.001$). Linear fits (R^2) ranged from 0.53 to 0.98. No significant slope differences were found between soil treatments after they were respiked with ^{14}C-pyrene at Day 110. Slopes of pooled data were 3.21 ± 0.29 and 0.43 ± 0.10 for Days 110 through 120 and 130 through 170, respectively.

	Linear mineralization phase†	
Soil treatment	**Days 1 through 7**	**Days 10 through 70**
Root extract-amended	**5.78* ± 0.13**	**0.37 ± 0.05**
Unamended	**4.96 ± 0.13**	**0.33 ± 0.05**
Excised root-amended	**4.70 ± 0.25**	**0.43* ± 0.03**

† Within columns, the significantly steepest slope is noted by an asterisk.

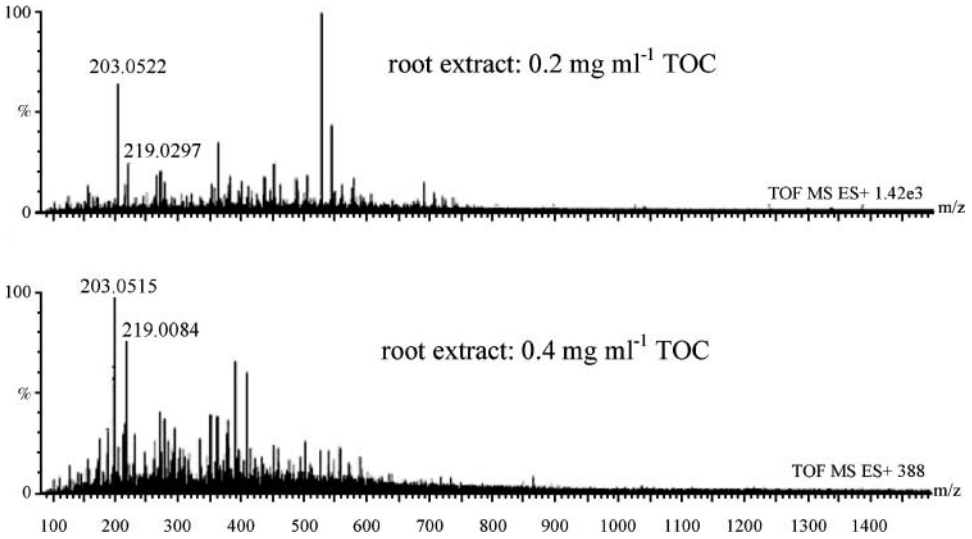

Fig. 4. Mass distributions found in extracts of red maple tree roots. The top panel shows the peaks generated from the electrospray ionization-mass spectrometry (ESI-MS) of an extract with a lower total organic carbon (TOC) (0.2 mg mL^{-1}) content, while the bottom panel is of an extract with twice that amount.

ments (see Materials and Methods); therefore, the excised root treatment certainly resulted in addition of higher overall levels of carbon substrates. Thus the nature of carbon present in root amendments was likely more important than the quantity in determining the observed effects on pyrene mineralization.

In this study, root extracts were also characterized in terms of the masses present in the amendment. The range of masses found in extracts (100 to 700 mass units) indicates a much more restricted composition than would be expected in a whole (or excised) fine root. The lower end of this range would consist of simple sugars, organic acids, and/or amino acids that could serve as growth substrates for pyrene degraders. The masses at the higher end of the range would encompass small secondary products and aromatic molecules known to be produced and often released by plants. The presence of these aromatic compounds in the rhizosphere may enhance microbial degradation of pyrene via cometabolism (Olson et al., 2001; Leigh et al., 2002; Rentz et al., 2005). Note that the extracts contained major peaks at 203 and 219 mass units (Fig. 4) which are very close in size to pyrene (molecular weight 202). Few peaks with mass units greater than 1000 were observed, which effectively excludes macromolecules and structural components such as starch, cellulose, lignin, and proteins from being present in the root extracts. These macromolecules would certainly be present when excised roots were added to soil to simulate fine root death and decay. The absence of these macromolecules is of note as other authors have suggested that root extracts mimic substrate release from fine root death and decay (Kamath et al., 2004; Rentz et al., 2004; Rentz et al., 2005).

Although the utility of using root extracts to study plant-microbe interactions is clear, very little is known regarding the exact composition of root extracts and their semblance to root exudates or root decomposition products. In previous studies that attempted characterization of root extracts, the constitution of greater than 90% of the TOC in root extracts was completely unknown in most cases (Kamath et al., 2004; Rentz et al., 2004). Our study narrows the range of possible compounds present in root extracts by showing that for both extracts analyzed the primary components fall within the same range of mass units. However, the mass and abundance of individual components within that range appear to be variable for each extract (Fig. 4) and it is unclear what proportion of this variability can be attributed to actual variation in the compounds present in plant roots or to the methods of extract production. Further characterization of root extracts prepared according to the methods used here (and other variations) would contribute greatly to interpretation of results attained by these and later studies.

A shortcoming of studies investigating the effects of root extracts or exudates on PAH degradation is the inability to capture temporally variable aspects of exudation including diurnal and seasonal variation that could influence root-microbe interactions in the field. The effect of actual root exudation on microbial mineralization of PAHs will be more variable than simulations such as ours indicate.

Previous studies have suggested that rhizosphere soil may be both nutrient-depleted (Wang et al., 2001; Wang et al., 2004) and nutrient-limited in terms of PAH degradation capacity (Joner et al., 2002; Siciliano et al., 2003). However, in this study inorganic nutrient amendment reduced cumulative pyrene mineralization relative to unamended rhizosphere soil. One possible explanation for this is that increased nitrogen and/or phosphorus availability in the rhizosphere could stimulate preferential utilization of more readily available carbon substrates provided by exudation or decay. Carmichael and Pfaender (1997) came to a similar conclusion after finding that addition of inorganic nutrients decreased mineralization of pyrene in several soils, but that mineralization of salicylic acid, amino acids, and glucose increased after nutrient amendment (also see Van der Krift et al., 2001).

In bulk soil, where such carbon sources are less available, increased nitrogen availability may stimulate degradation of the relatively more abundant PAH. In our study pyrene mineralization in bulk soil was initially increased by a single amendment with nitrogen and phosphorus but the final extent of pyrene mineralization in nutrient-amended and unamended soils was not significantly different, suggesting that in bulk soils the effect of inorganic nutrients on POP degradation may be short-lived without continuous application. Increased PAH mineralization in bulk soil has also been reported as a result of nutrient amendment in phytoremediation field trials (Siciliano et al., 2003). The effect of inorganic nutrient amendments may also depend on the nutrient status of the soils in question. Johnson and Scow (1999) reported that nutrient additions decreased phenanthrene mineralization in soils with low nutrient levels but did not affect soils with higher levels of soil nutrients, particularly phosphorus.

CONCLUSIONS

Our results and recent work by others (Genney et al., 2004; Kamath et al., 2004; Qiu et al., 2004; Rentz et al., 2004) demonstrate that plant-microbial interactions in the rhizosphere will not always have wholly positive effects on organic contaminant degradation. A better understanding of the controls of plant-microbial interactions in the rhizosphere (including organic and inorganic nutrient availability) is needed to appropriately select plants and soils for applications of phytoremediation. Incorporating the effects of seasonal variation will be a key element in accurately extrapolating the results of phytoremediation trials to more realistic settings.

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